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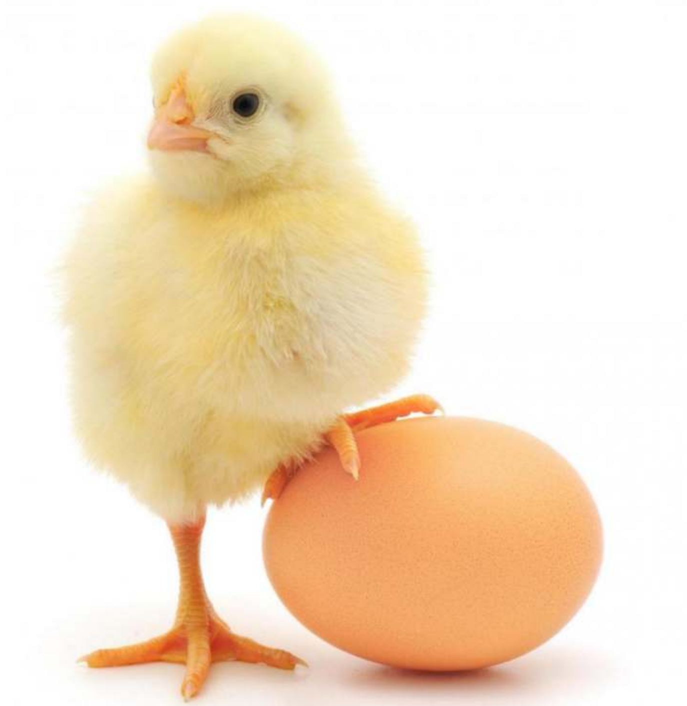

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Effects of Dietary *Aspergillus* Meal Prebiotic on Turkey Poults Production Parameters and Bone Qualities†

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Abstract: The objective of this study was to evaluate the effects of 0.2% dietary *Aspergillus* Meal (AM) on performance and bone parameters of neonatal turkey poults. A total of 200 day-of-hatch turkey poults were used for this experiment. Two dietary treatments, similar in energy and protein content differing only by the addition of 0.2% AM, were used. Poults were divided into 2 treatment groups with 25 birds per treatment and four replicates each. Group 1 received a basal non medicated control diet and group 2 received dietary AM. At the end of 30 d, poults were weighed, euthanized, and tibias were collected to evaluate bone quality using an Instron shear press machine and bone parameters such as tibia weight, diameter, ash, calcium and phosphorus assays. Samples of distal ileum were collected and the content subjected to protein and energy analysis. Poults fed with dietary AM had a significant improvement in BW and feed conversion ratios ($p<0.05$). Distal ileum content showed significantly less concentration of energy and protein when compared with the poults receiving control diet. Tibia weight, diameter, breaking strength, ash, calcium and phosphorus were significantly higher in poults that received dietary AM prebiotic. These results suggest that the increase in performance and bone parameters in neonatal turkey poults fed with 0.2% AM, is improved upon feeding *Aspergillus niger* mycelium prebiotic.

Key words: *Aspergillus* meal, prebiotic, turkeys, productive parameters, bone qualities

INTRODUCTION

According to the definition by Gibson and Roberfroid (1995), prebiotics are "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health". Some prebiotics have been shown to selectively stimulate the growth of endogenous lactic acid bacteria in the gut thereby improving the health of the host (Gibson and Wang, 1994). Thus, prebiotics can selectively modify the colonic microflora and potentially influence gut metabolism (Laparra and Sanz, 2009). The presence of normal gut microflora may improve the metabolism of host birds in various ways including absorptive capacity (Yokota and Coates, 1982), protein metabolism (Salter *et al.*, 1974), energy metabolism, fiber digestion (Furuse and Yokota, 1984) and gut maturation (Furuse *et al.*, 1991). A healthy population of these beneficial bacteria in the digestive tract enhances the digestion and absorption of nutrients, detoxification, elimination processes and helps boost the immune system (Chow, 2002; Tokunaga, 2004; No *et al.*, 2007; Kong *et al.*, 2010). The commercially available fermentation product of *Aspergillus niger*, Fermacto®, referred to as *Aspergillus* Meal (AM), has no live cells or spores and is proven to enhance the digestive efficiency of the gut (Potter and Shelton, 1984; Harms and Miles, 1988). Prebiotics have been shown to stimulate Calcium (Ca) and Magnesium (Mg) absorption in the intestine and increase bone mineral concentrations in humans and rats (Tokunaga, 2004; Abrams *et al.*, 2007; Lobo *et al.*, 2009). However, the effects of prebiotic feed supplements on bone development in poultry are lacking. The objective of this study was to evaluate the effects of dietary AM prebiotic on performance and bone parameters of neonatal turkey poults.

MATERIALS AND METHODS

Diet composition and preparation: Control and treated poults were fed a corn-soybean starter diet. The diets were formulated without added antibiotics or coccidiostats and contained levels of nutrients recommended by the National Research Council (1994). The two treatments, similar in energy and protein content, differed only by the presence of 0.2% of the

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prebiotic, AM, a dried primary fermentation AAFCO/GRAS (AAFCO, Inc., 2011) *Aspergillus niger* Strain (Fermacto®, PetAg Inc. Hampshire, IL60140USA). This mycelium is unique because it contains only 16% protein and 45% fiber (Harms and Miles, 1988).

Experimental design: A total of 200 day-of-hatch turkey poults were used for this experiment. Poults were divided into 2 treatment groups with 25 birds per treatment (four replicates each) and received either a basal non medicated control diet or the same diet with AM prebiotic. All poults were fed for 30 days. At the end of 30 days, body weight, feed conversion and mortality were recorded and all poults were euthanized. Tibias from five pouts in each replicate were collected to evaluate bone qualities. Samples of ileum were also collected from the same birds and their content subjected to protein and energy analysis.

Distal ileum content analysis: Ileal sections (from Meckel's diverticulum to the ileo-caecal junction) were taken after sacrificing the poults. The ileal content was collected and then frozen. Nitrogen content was determined with an automatic analyzer (Leco FP-528 nitrogen, Leco Corp., St Joseph, MI) by AOAC 968.06 procedure (AOAC International, 2000) using EDTA as the standard and the protein content was calculated as nitrogen x 6.25. Gross energy in the ileum content was determined with an adiabatic bomb calorimeter (model 1261 isoperibol, Parr Instrument Co., Moline, IL) using analytical grade sucrose as the standard. Crude protein and gross energy were determined in triplicate samples.

Bone parameters: Bone parameters were measured according to the methods described by Zhang and Coon (1997). Tibias from each poult were cleaned of attached tissues. Bones from the right leg were subjected to conventional bone assays and tibia from the left leg were used to determine breaking strength.

Conventional bone assays: The bones from right tibia and femurs were dried at 100°C for 24 h and weighed again. The bones were subsequently ashed at 600°C overnight, cooled in a desiccator and weighed. The samples were then ashed in a muffle furnace (Isotemp muffle furnace, Fisher Scientific, Pittsburgh, PA) at 600°C for 24 h in crucibles. Finally, the content of calcium and phosphorus in the tibia was determined using standard methods (AOAC International, 2000).

Bone breaking strength: Bone breaking strength was measured using an Instron shear press with a 50-kg load cell at 50-kg load range with a crosshead speed of 50 mm/min; bone was supported on a 3.00-cm span (Huff *et al.*, 1980).

Statistical analysis: All data were subjected to one-way analysis of variance as a completely randomized design using the General Linear Models procedure of SAS (SAS Institute, 2002). Significant differences among the means were determined by using Duncan's multiple-range test at $p<0.05$.

RESULTS AND DISCUSSION

Prebiotics are nondigestible food ingredients containing oligosaccharides that are selectively fermented by one or more bacteria known to have positive effects on gut physiology. Bacteria fed with a preferential food substrate have a proliferative advantage over other bacteria (Gibson and Wang, 1994). *Aspergillus* fiber contains beta-glucans(McCleary and McCleary, 2000), Fructo-Oligosaccharides (FOS) (Sangeetha *et al.*, 2004), chitosan (Jonker *et al.*, 2010; Muzzarelli, 2010) and Mannan-Oligosaccharides (MOS) (Uchima *et al.*, 2011; Vera *et al.*, 2011). Beta-glucan is a powerful immune-enhancing nutritional supplement. It affects the intestinal villi and primes the innate immune system to help the body defend itself against viral and bacterial invaders (Tsukada *et al.*, 2003; Lowry *et al.*, 2005; Jonker *et al.*, 2010). MOS protect the GI tract from invading toxins by binding the toxin active sites (Biggs *et al.*, 2007). FOS and chitosan refer to a class of host non-digestible carbohydrates that are readily fermented by the beneficial bacteria in the intestine. A healthy population of these beneficial bacteria in the digestive tract enhances the digestion and absorption of nutrients, detoxification and elimination processes and helps boost the immune system (Chow, 2002; Tokunaga, 2004; No *et al.*, 2007; Kong *et al.*, 2010).

Previously, we have shown that dietary AM induces important changes in the intestinal morphometry in neonatal turkey poults, suggesting that AM prebiotic has a beneficial impact on the mucosal architecture and goblet cells proliferation (Tellez *et al.*, 2010). In the present study, dietary AM prebiotic supplemented for 30 days, significantly increased the body weight of neonate poults and improved feed conversion compared with poults that received only the control basal diet (Table 1). The energy and protein content in the ileum was significantly lower in poults that received dietary AM prebiotic compared with control poults suggesting better digestibility and absorption of those nutrients (Table 2). These results are in agreement with the morphometric changes observed previously (Tellez *et al.*, 2010). Table 3 summarizes the effect of AM on bone breaking strength and other bone parameters (variables) in turkey poults at 30 d of age. Significant increases in tibia weight, diameter, breaking strength, ash, calcium and phosphorus were observed in poults that received dietary AM when compared with neonatal poults that received the control basal diet. FOS has been shown to stimulate Calcium (Ca) and Magnesium (Mg) absorption in the intestine and increase bone mineral concentrations in humans and rats (Tokunaga, 2004; Abrams *et al.*, 2007; Lobo *et al.*, 2009). Dietary short-

Table 1: Effect of *Aspergillus* meal on productive parameters in turkey poults at 30 days of age

	Control	*Aspergillus* meal
Body weight (kg)	600.32±52.26[b]	720.87±63.82[a]
FC (Feed: gain)	1.34±0.029[a]	1.23±0.023[b]
Mortality (%)	2.0%[a]	2.5%[a]

A total of 200 day-of-hatch turkey poults were used for this experiment. Poults were divided into 2 treatment groups with 25 birds per treatment (four replicates each) and received either a basal non medicated control diet or the same diet with AM prebiotic. All poults were fed for 30 days. At the end of 30 days, body weight, feed conversion and mortality were recorded.
Data is expressed as mean±standard error. Values within a row with no common superscript differ significantly $p<0.05$. FC = Feed Conversion

Table 2: Effect of *Aspergillus* meal on chemical proximal analysis of distal ileum content in neonatal turkey poults at 30 days of age

	Control	*Aspergillus* meal
EC (Calories/g)	425.00±28.21[a]	250.00±31.45[b]
Protein (%)	3.00±00.61[a]	1.23±00.78[b]

Ileum samples from five pouts in each replicate were collected and their content subjected to protein and energy analysis.
Data is expressed as mean±standard error. Values within a row with no common superscript differ significantly $p<0.05$. EC = Energy Content

Table 3: Effect of *Aspergillus* meal on bone breaking strength and bone parameters of neonatal turkey poults

	Control	*Aspergillus* meal
Tibia weight (g/100 BW)	0.85±0.02[b]	0.90±0.009[a]
Tibia strength (kg force)	0.14±0.011[b]	0.18±0.009[a]
Tibia diameter (mm)	4.10±0.17[b]	4.61±0.28[a]
Total ash from tibia (%)	45.01±0.41[b]	48.87±0.35[a]
Calcium (% of ash)	35.48±0.27[b]	39.48±0.27[a]
Phosphorus (% of ash)	17.15±0.12[b]	20.15±0.12[a]

Tibias from five pouts in each replicate were collected to evaluate bone qualities. Samples of ileum were also collected from the same birds and their content subjected to protein and energy analysis.
Data is expressed as mean±standard error. Values within a row with no common superscript differ significantly $p<0.05$

chain FOS increases calbindin-D9k levels in rats (Takasaki *et al*., 2000). Mineo *et al*. (2001) reported that FOS stimulates net Ca transport from the epithelium of the small and large intestine of rats *in vitro* (Mineo *et al*., 2001). The gastrointestinal tract serves as the interface between diet and the metabolic events. Intestinal villi, play a crucial role in digestion and absorption of nutrients, are underdeveloped at hatch (Uni *et al*., 1995) but obtain maximum absorption capacity by 10 days of age (Uni *et al*., 1996). Understanding and optimizing the maturation and development of the intestine in poultry may improve feed efficiency, growth and overall health of the bird. Studies on nutrition and metabolism during the early phase of growth in poults may, therefore, help in optimizing nutritional management for maximum growth (Mahagna *et al*., 1995). By dietary means it may be possible to positively affect the development of the gut and provide the competitive advantage in favor of beneficial bacteria which can alter not only gut dynamics, but also many physiologic processes due to the end products metabolized by symbiotic gut microflora. Additives such as enzymes, probiotics and prebiotics are now extensively used throughout the world. The chemical nature of these additives are better understood but the manner by which they benefit the animal is not clear (Chow, 2002; Schneeman, 2002; de Vrese and Schrezenmeir, 2008). The results of this study suggest that the increase in performance and bone parameters in neonatal poults fed with 0.2% AM, may be related to a synergistic effect between beta-glucan, MOS, chitosan and FOS from *Aspergillus niger* mycelium.

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